Yeast Protein Extracts

This protocol is a simple, reliable method for preparation of yeast protein extracts for PAGE analysis and Western blotting. This procedure works for both growing and stationary phase cells, grown either in liquid or on plates (YPD or minimal). A sample of 2.3 mg (wet weight) of cells yields sufficient protein to run several lanes on a minigel. The technique was adapted from Kushnirov (2000).

PROCEDURE

1. Collect about 2.5 OD_{600} of cells (approximately 2.3 mg wet weight) from a liquid culture or scraped from the surface of a plate with a bacteriological loop. Resuspend the cells in 100 µl of distilled water, add 100 µl of 0.2 M NaOH, and incubate the sample for 5 minutes at room temperature.
2. Pellet the sample, and resuspend it in 50 µl of PAGE sample buffer. Boil the sample for 3 minutes and pellet again. Use a pipette to remove the supernatant, which contains protein. Use 6 µl of the supernatant per lane and resolve on a PAGE minigel.
3. To measure protein concentration by Bradford assay, dilute the sample 1:3000.

MATERIALS AND SOLUTIONS

0.2 M sodium hydroxide solution
2x PAGE sample buffer
120 mM Tris/HCl (pH 6.8)
10% glycerol
4% SDS
8% β-mercaptoethanol (I use 5%)
0.004% bromophenol blue

REFERENCE

Kushnirov V.V. 2000. Rapid and reliable protein extraction from yeast. *Yeast* **16:** 857–860.